PHYTOPLANKTONS AS BIOINDICATOR FOR THE ASSESSMENT OF WATER EFFLUENTS OF AMANISHAH NALA, JAIPUR.

Research Protocol
For Registration for Ph.D. Programme
Submitted by
RITU SINGH RAJPUT

Under the Guidance of
Dr. Sonali Pandey
Associate Professor,
Department of Botany

and

Dr Seema Bhadauria
Assistant Professor
Department of Microbiology

2014
JECRC UNIVERSITY, JAIPUR

CONTENTS

1. INTRODUCTION

2. PART LITERATURE REVIEW

3. STATEMENT OF THE RESEARCH PROBLEM

4. OBJECTIVES OF THE STUDY

5. SCOPE OF THE STUDY

6. SIGNIFICANCE OF THE STUDY

7. LIMITATIONS

8. METHODOLOGY

9. MONTH WISE PLAN OF WORK

10. MONTH WISE PLAN OF WORK (GRAPHICAL REPRESENTATION)

11. REFERENCES

INTRODUCTION

Industrialization is the period of communicative and economic exchange that transforms a human group from a rural society into an industrial one. It is a part of a wider modernization procedure, where social change and economic development are closely related with technological innovation, particularly with the growth of large-pane resolution and metallurgy production. It is the wide organization of an economizing for the purpose of manufacturing. Pollution is the introduction of contaminants into the natural environment that causes adverse change. Pollution can take the form of chemical substances or energy, like as noise, heat or light. Pollutants, the components of pollution, can be either foreign substances/energies or naturally occurring contaminants as-

(1) Air pollution: - the release of chemicals and particulates into the atmosphere. Some gaseous pollutants include carbon monoxide, sulfur dioxide, chlorofluorocarbons (CFCs) and nitrogen oxides produced by industry and engine vehicles. Photochemical ozone and smog are created as nitrogen oxides and hydrocarbons react to sunlight.

(2) Soil contamination appears when chemicals are quit by help or underground leakage. Among the most significant soil contaminants are hydrocarbon, heavy metal, herbicides, pesticides and chlorinated hydrocarbons (Bath ,1989).

(3) Water pollution, by the discharge of wastewater from commercial and industrial waste (intentionally or through spills) into surface waters; discharges of untreated domestic sewage, and chemical contaminants, such as chlorine, from treated sewage; release of waste and contaminants into surface runoff flowing to surface waters waste disposal and leaching into groundwater; eutrophication and littering (Howells and Gwyneth,1994) .

Water is an important resource because all living organisms require it to survive. Polluted water poisons plants and animals, and has a direct impact on humans. The main causes of water pollution are sewage, industrial wastes, oil spills and the use of pesticides in agriculture. When untreated sewage is emptied into rivers, it causes diseases like typhoid, dysentery and cholera. Algae grow uncontrollably, using the oxygen in the water and causing the death of all the water organisms. Harmful bacteria flourish in untreated sewage. When drinking and

swimming water becomes contaminated, the disease-causing bacteria spread rapidly, making many people ill. Many industrial processes produce toxic wastes. In some cases, toxic wastes are illegally pumped into rivers, streams and dams. Chemicals like cyanide, mercury, lead and copper, are non-biodegradable. Organisms are poisoned as the concentrations of toxins increase, and are passed through the food chain. The poisonous chemicals cause cancer and damage internal organs in people and animals. Factories and power stations sometimes pump hot water into rivers and streams causing thermal pollution. Increased temperatures kill the water plants and the fish because fish are cold-blooded and cannot tolerate extreme changes of environmental temperature (Xu, S and Nirmalakhandan, 1998).

Algae are photosynthetic organisms that occur in most habitats, ranging from freshwater and marine and from hot boiling springs to snow and ice. They vary from small, single-celled forms to complex multicellular forms, such as the giant kelps of the eastern Pacific that grow to more than 60 meters in length and form dense marine forests. Algae are important as primary producer of organic matter at the base of the food chain. They also provide oxygen for other aquatic life. Algae may contribute to mass mortality of other organisms, in cases of algal blooms, but they also contribute to economic well- being in the form of food, medicine and other products. In tropical regions, coralline algae can be as important as corals in the formation of reefs.

Algae are the main the primary producers in all kinds of water bodies and they are involved in water pollution in a number of significant ways. Firstly, enrichments of the algal nutrients in water through organic effluents may selectively stimulate the growth of algal species producing massive surface growths or „blooms" that in turn reduce the water quality and affect its use. However certain algae flourished in water polluted with organic wastes play an important part in "self-purification of water bodies". In fact, algae can play significant part of food chain of aquatic life. Algae are also known to be causes of tastes and odors in water (Sigworth ,1957). Certain diatoms, blue-green algae and coloured flagellates (particularly *Chrysophyta* and *Euglenophyta*) are the best known algae to pose such problems in water supplies, but green algae may also be involved (Silvey *et .al*, 1959).

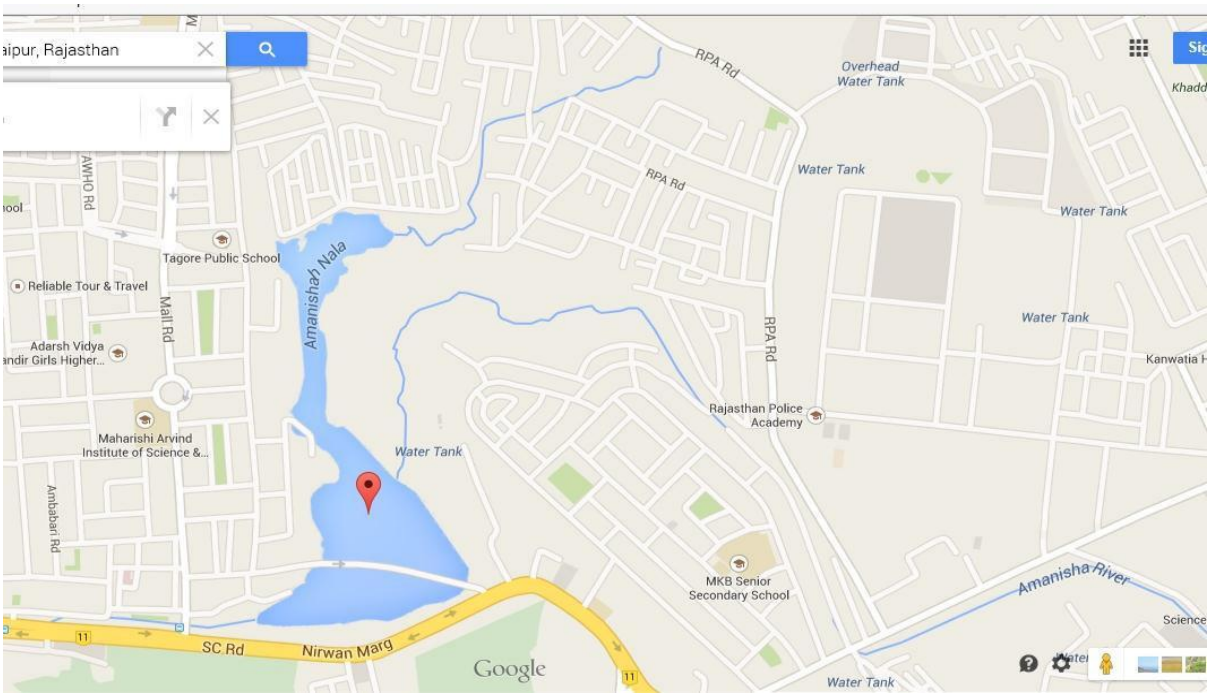
Bio-indicators can be defined as an organism that provides quantitative information on the quality of environment around it. Bio-indicator organisms can be used to identify and qualify the effects of pollutants on the environment. Bio-indicators can

say to us about the total amount of effects of different pollutants in the ecosystem and about how long a problem may persist. Algae of many kinds are really good indicators of water quality and many lakes are represented based on their dominant phytoplankton group (Brook, 1965). Similarly, many blue-green algae occur in nutrient-poor waters, while some grow well in organically polluted waters. (Paswan and Kumar, 2014). Algae are most useful as indicators in the context of eutrophication but have been employed as well to detect organic pollution because of their well documented tolerance.(Palmer ,1969).Their value as bio-accumulators of e.g. pesticides are heavy metals is limited. Although indicator organisms can be any biological species that defines a trait or characteristics of the environment, algae are known to be good indicators of pollution of many types for the following reasons.

1. Many algal species are available all the year.
2. Algae are diverse group of organisms found in large quantities.
3. Response quickly to the charges in the environment due to pollution.
4. Easier to detect and sample.
5. The presence of some algae are well correlated with particular type of pollution Particularly to organic pollution

Algae are a very diverse group of organisms. This natural succession of algae occurs in response to changes in season, temperature, wind, precipitation patterns, and nutrient cycles. (Moore and Thornton, 1988) .Algal populations are abundant in spring and early summer when available light and nutrients are high and few organisms are present to feed on the algae. Toward the end of this stage, an appearance, usually known as the clear water phase, appear in many lakes, ponds. Spring algal populations are mainly composed of small, highly edible species. The small, edible algal populations are gradually replaced by larger, colonial, non-edible species that are often covered by gelatinous sheaths. Because present nutrient concentrations are often limited in the summer, the total concentration of algae in summer can be less than in spring before the clear water phase. In late summer and fall, nutrients stored at the bottom of the lake become mixed through the water column generating a fresh supply of nutrients. This allows algal populations to flourish again and late season algal blooms to develop. During winter months algae are able to survive, but usually at low concentration due to colder water temperature and lower amounts of available sunlight.

When just 55% of Jaipur was connected by sewers and most waste discharged directly into the Amani Shah Nala, the once majestic Dravavati River was nowhere to be seen. Today, sewerage is up to 75% and sullage connectivity up from 35% to 75%.Prior to 1874, water was obtained through open wells, and channels from which water was cool in kunds and the metropolis allege a relatively sufficient water supply. However, with augmentation of Jaipur through the 1700s and 1800s, enlargement of the irrigate supply scheme to ensure a sustained water supply was required. In 1844 a dam was build across Amanishah Nala, but it was breached in 1853. At the opportunity of the dam construction, Amanishah Nala was a perennial nala. However, after some years, the inflow of water into the Amanishah Nala became low in fiery seasons. Heavy silting of the dam also contributed to a reduction in the faculty of Amanishah Nala. Open wells and tube wells were subsequently sunk into this reservoir, and water was pumped from these wells to assert water yield. This Amanishah water system did on with the earlier system of water canals and the collection of water in kunds. The soon water occupy system of Jaipur initially relied upon groundwater, but the topography of Jaipur was considered and thus the sewage patterns allowed for recharge of groundwater. Amanishah Nala later provided a surface water source involving the transportation of water through a canal and the collection of this water into kunds, though groundwater supplied the majority of water supply. (Roberts, et.al, 2013)



AMANISHAH NALA

PART LITERATURE REVIEW

Water is an elixir of life. It governs the evolution and function of the universe on the earth hence water „mother of all living world". Majority of water available on the earth is saline in nature; only small quantity is fresh water. Freshwater has become a scare commodity due to over exploitation and pollution. Pollution is caused when a change in the physical, chemical or biological condition in the environment harmfully affect quality of human life including other animals" life and plant. Industrial, sewage, municipal wastes are been continuously added to water bodies hence affect the physiochemical quality of water making them unfit for use of livestock and other organisms. Uncontrolled domestic waste water discharge into pond as resulted in eutrophication of ponds as evidenced by substantial algal bloom, dissolve oxygen depletion in the subsurface water leads to large fish kill and other oxygen requiring organism (Muhibbu-Din, *et.al*, 2011).

Algae are a very large and diverse group of simple typically autotrophic organisms, ranging from unicellular to multicellular forms, such as the giant kelp (large brown alga), that may grow up to 50 meters in length. Most are photosynthetic and "simple" because they lack many of the distinct cell organelles and cell types found in land plants. The largest and most complex marine forms are called seaweeds.

Palmer surveyed microalgae genera from a wide distribution of waste stabilization ponds. In order of abundance and frequency of occurrence the algae found were *Chlorella*, *Ankistrodesmus*, *Scenedesmus*,*Euglena*, *Chlamydomonas*, *Oscillato ria*, *Micractinium* and *Golenkinia*.(Palmer, 1969).

Potamogeton perfoliatus, Eichhornia crassipes, and Microystis aeruginosa has been observed to grow frequently in Lake Udaisagar (Prakash, and Vijayvergia, 2008). *Microcystis and spirulina (cynophycea), Cycolatella, Navicula* and *Nitzschia (Bacillariophyceae),* and *Chlorella, Ankistrodesmus (Chlrophyceae) has been* observed in Mansager Lake (Sharma,*et.al,*2007).*Microalgae* strain *Chlorella minutissima*, *BGA (Nostoc) & Scendesmus spp* was obtained IARI"s sewage (New Delhi, India),(Sharma,*et.al*,2013).Green microalgae *Chlorella vulgaris* species was collected from Bhavani Lake at Erode District, Tamil Nadu State, India(Ponnuswamy,2013). The simple and common green algae genus *chlorella* is

placed below the order.*Chlorococcales* and family *Chlorellacese* (Hoek and Mann, 1998).

Microalgae Chlorella sp. are known to used in the treatment of wastewater, production of biodiesel, production of electricity using microbial fuel cells, animal food supplements and providing valuable extracts for chemical products (Phang Siew Moi,2004), (Chisti and Yusuf ,2007).

Some of the more common pollution-tolerant genera encountered in Washington water bodies include: *Chlamydomonas, Desmodesmus* (*Scenedesmus* with spines), *Euglena*,*Lepocinclis* (some *Euglena,Phacus), Nitzschia, Oscillatoria, Pandor ina, and Phacus.* Toxic *cyanobacteria* are found worldwide in inland and coastal water environments. At least 46 species have been shown to cause toxic effects in vertebrates . The most common toxic cyanobacteria in fresh water are *Microcystis* spp., *Cylindrospermopsis raciborskii*, *Planktothrix* (syn. *Oscillatoria*) *rubescens*, *Synechococcus* spp., *Planktothrix* (*syn. Oscillatoria*) *agardhii*, *Gloeotrichia spp., Anabaena spp., Lyngbya spp., Aphanizomenon spp., Nostoc spp*., some *Oscillatoria* spp, *Schizothrix* spp. and *Synechocystis* spp.(Duy,*et.al*, 2000).

The algae inoculums collected from the waste stabilization pond is identified, and the prominent genera found in the sample are *Anabaena, Diatoms*, *Hyalophacus Monoraphidium, Spirogyra*. (Velan and Saravanane ,2013).

Five algal species were selected as indicators of the degree of pollution in rivers in England. *Stigeoclonium tenue* is present at the downstream margin of the heavily polluted part of a river, *Nitzschia palea and Gomphonema parvulum* always appear to be dominant in the mild pollution zone whilst *Cocconeis* and *Chamaesiphon* are reported to occur in unpolluted parts of the stream or in repurified zone.(GUPTA and Kumar,2014). *Navicula accomoda* is stressed to be a good indicator of sewage/organic pollution as the species comfortably occur in the most heavily polluted zones in which other species cannot occur. The same hold true for species and varieties of *Gomphonema* (Archibald,1972).*Amphora ovalis* and *Gyrosigma attenuatum* are also introduced as good examples of diatoms to be affected by high organic content of water.(Patrick ,1948).

STATEMENT OF THE RESEARCH PROBLEM

Phytoplanktons as Bio-indicator for the assessment of water effluents of Amanishah Nala, Jaipur.

OBJECTIVES

1. To collect the water sample in different seasons (summer, winter, rainy) from Amanishah Nala, Jaipur (from different location in jaipur) throughout the year.
2. To analysis the physiochemical parameters of water samples.
3. To identify the algal species from seasonal water samples by microscopic methods.
4. Identification of algal species as bio-indicator.
5. Molecular characterisation of algal species as bio indicator

SCOPE OF THE STUDY

1. Collection of water samples in different seasons from Amanishah Nala Jaipur and analysis of physiochemical properties.
2. Identification of algal species and its correlation with water quality.

SIGNIFICANCE OF THE STUDY

1. Algae are valuable indicators of ecosystem conditions because they respond quickly both in species composition and densities to a wide range of water conditions due to changes in water chemistry.
2. When pollution occurred in Amanishah nala, it gives early warning sign like increase their temp, pH, BOD, COD. So we can easily identify the pollution.

LIMITATIONS

1. It is site and season specific.
2. Although a large number of bio-indicators are reported but the present study will be limited only on phytoplanktons

METHODOLOGY

These are some steps of methodology.

Microalgae have a key role in bio monitoring the changes that occur in aquatic habitats not only because they are the main primary producers of new organic substances in these ecosystems, but also because of their pronounced physiological plasticity that supports their acclimation to changes in the physical and chemical properties of the environment

1. Algal Sampling, Identification and Enumeration:-The samples will be collected from AMANI SHAH NALA, in different seasons (summer, winter, rainy). The water sample will be collected in the sterilized glass bottles. These sample bottles will be rinsed three times with source water to minimize the risk of external contamination before sampling. Samples for the investigation of phytoplankton will be collected randomly from site. Algae will be identified and counted microscopically. 100 ml water sample will be collected from the selected locations. 15 ml samples will take and centrifuged at 3000 rpm at room temperature. The concentrate, ~20 µl of the sample will take onto glass slide, counted and enumerated under light microscope (40 X). Representative images were taken at 100 X magnification.The identification of algal species will be carried out based on the morphological features, unicellular, filamentous, colour, motality, and reproductive structures(Prescott,1956).

2. Physico-Chemical Parameters: - Microalgae have a key role in bio monitoring the changes that occur in aquatic habitats not only because they are the main primary producers of new organic substances in these ecosystems, but also because of their pronounced physiological plasticity that supports their acclimation to changes in the physical and chemical properties of the environment (Fodorpataki, L.,Bartha ,2008).Physico- chemical parameter study is very important to get exact idea about the quality of water and we can compare results of different physico chemical parameter values with standard values. It is very essential and important to test the water before it is used for drinking, domestic, agricultural or industrial purpose. Water must be tested with different physic-chemical parameters. Selection of parameters

for testing of water is solely depends upon for what purpose we going to use that water and what extent we need its quality and purity. Water does content different types of floating, dissolved, suspended and microbiological as well as bacteriological impurities. Some physical test will be performed for testing of its physical appearance such as temperature, color, odour, pH, turbidity, TDS etc, while chemical tests will be perform for its BOD, COD, dissolved oxygen, alkalinity, hardness and other characters analysed by using standard methods (Trivedi,*et*
.al,1986). For obtaining more and more quality and purity water, it will be tested for its trace metal, heavy metal contents and organic.

Table 1: Different analytical water quality parameters with their analytical technique and guideline values as per who and Indian standard (Patil *et, al* 2012).

Sr. No.	Parameter	Technique used	WHO standard	Indian Standard	EPA guidelines
01	Temperature	Thermometer	-	-	-
02	Color	Visual / color kit	-	5 Hazen units	-
03	Odour	Physiological sense	Acceptable	Acceptable	-
04	Electrical conductivity	Conductivity meter / Water analysis kit	-	-	2500 us/cm
05	pH	pH meter	6.5 – 9.5	6.5 – 9.5	6.5 – 9.5
06	Dissolved oxygen	Redox titration	-	-	-
07	Total Hardness	Complexometric titration	200 ppm	300 ppm	< 200 ppm
08	Alkalinity	Acid – Base titration	-	200 ppm	-
09	Acidity	Acid – Base titration	-	-	-
10	Ammonia	UV Visible Spectrophotometer	0.3 ppm	0.5 ppm	0.5 ppm
11	Bi carbonate	Titration	-	-	-
12	Biochemical Oxygen Demand (B.O.D.)	Incubation followed by titration	6	30	5
13	Carbonate	Titration	-	-	-
14	(C.O.D.)	C.O.D. digester	10	-	40
15	Chloride	Argentometric titration	250 ppm	250 ppm	250 ppm
16	Magnesium	Complexometric titration	150 ppm	30 ppm	
17	Nitrate	UV Visible Spectrophotometer	45 ppm	45 ppm	50 mg/l
18	Nitrite	UV Visible Spectrophotometer	3 ppm	45 ppm	0.5 mg/l
19	Potassium	Flame Photometer	-	-	-
20	Potassium	Flame Photometer	-	-	-
21	Sodium	Flame Photometer	200 ppm	180 ppm	200 ppm

3. Molecular techniques: - The polymerase chain reaction (PCR) is a biomedical technology in molecular biology used to amplify a single copy or a few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence. There we will study with the help of PCR amplification of specific nucleotides regions like 16s or 18s rRNA genes.16s rRNA present in Prokaryotes and 18s r RNA in Eukaryotes.

This technique has been used for example, to determine the taxonomic composition of bio films – Blue green algae and some eukaryote unicellular algae (Droppo, et.al, 2007).

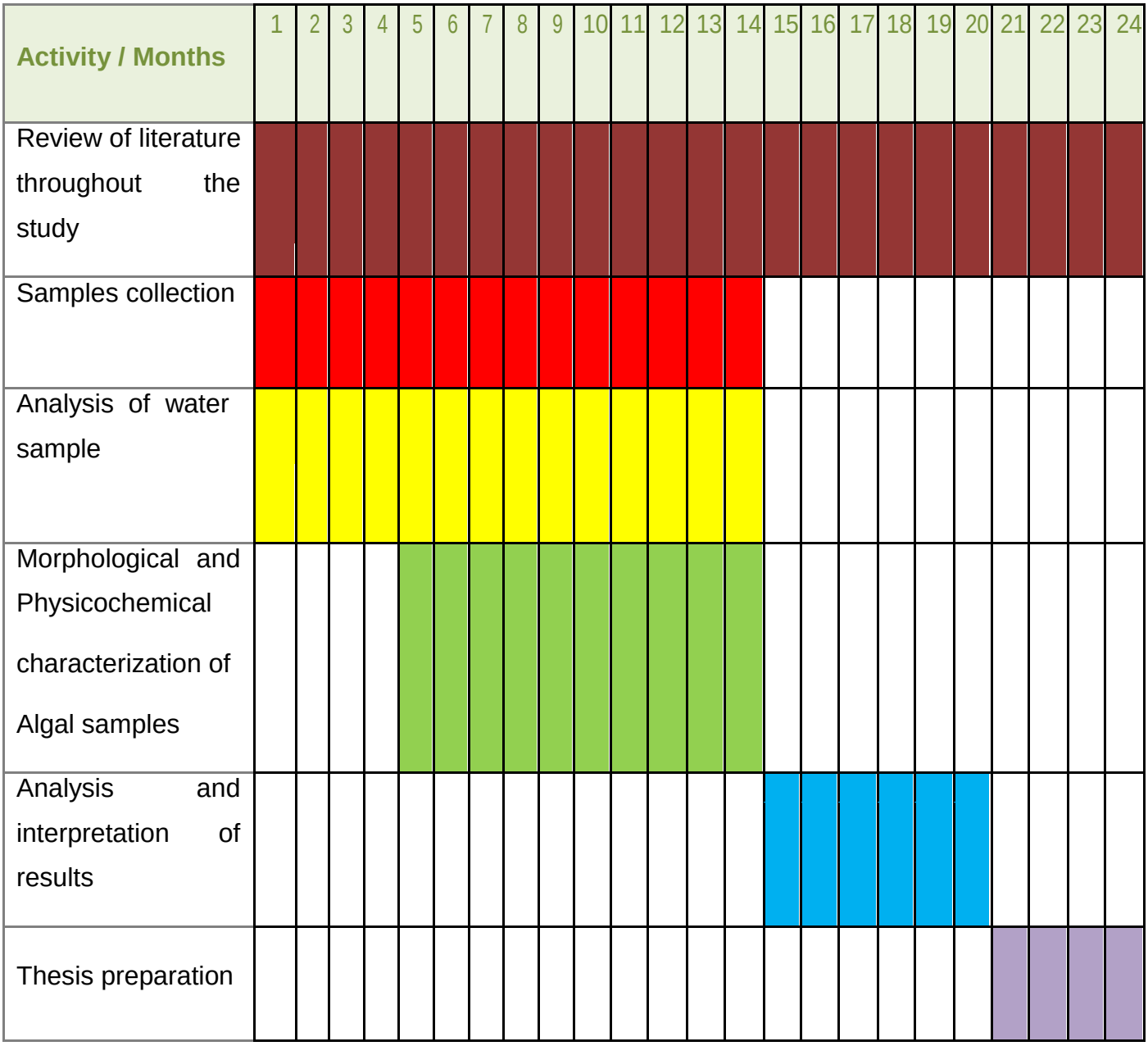
Environmental change is caused by an environmental stress factor .The influx of inorganic nutrients into a previously low-nutrient system. The resulting loss or dominance of particular bio indicator species is preceded by biochemical and physiological changes in the algal community referred to as „biomarkers (Bellinger, et, al, 2010). The algae species and amounts can directly reflect the water quality. Heavy metal exposure can cause the disturbance of normal metabolism and biological function, inhibition of photosynthesis, reduction of cytochrome, cellular mutation, putrescence, even death in algae. More importantly, once heavy metal pollutants are accumulated in these organisms, they enter the food chain and may pose serious threaten to animals and human health through biomagnifications. Water pollution type and level can be accurately identified by analyzing the species, amounts, physiological and biological responses and residue contents. Algae may not only be significant for bio monitoring studies and could also be a useful phytoremediation technology to restore water quality due to high bioaccumulation abilities.

MONTH WISE PLAN OF WORK

1-24 months	Review of literature throughout the study
1-14 months	Samples collection
1-14 Months	Analysis of water sample
1-14 Months	Morphological and physicochemical ,characterization of Algal samples
1-14 months	Analysis and interpretation of results
20-24 months	Thesis preparation

Total Time (Expected): 24 Months

MONTH WISE PLAN OF WORK (GRAPHICAL REPRESENTATION)



REFERENCE LIST

1. Archibald, .E (1972) Diversity in some South Africation diatom associations and its Relation to water quality *water Res,* Vol (6), pp.1229-1238.

2. Baath, E. (1989) "Effects of heavy metals in soil on microbial processes and populations‟ .Water, Air, and Soil Pollution. *Kluwer Academic Publisher*, Vol (47), 335-379, [online] DOI: 10.1007/BF00279331 (Accessed: 1 october 1989).

3. Bellinger. Edward. G. and David, C.S. (2010)"Introduction to Freshwater Algae."Freshwater Algae: Identification and Use as Bioindicators. *Publisher John Wiley&Sons,Ltd,Chichester,andUK.pp.40*[online]DOI: 10.1002/9780470689554.ch1, (Accessed: 12 Apr 2010).

4. Brook, A. J (1965) Planktonic algae as indicators of lake types, with special reference to the Desmidiaceae. Limnol and Oceannog*, Limnology and Oceanography Publication*, Vol 10(3), pp 403-411.

5. Chisti and Yusuf (2007)" Biodiesel from microalgae." Biotechnology advances, *Elsevier publication*, vol 25(3), pp.294-306.

6. Droppo, l. G, Nathalie, R. Skafel, M. and Steven, N. L. (2007), Biostabilization of cohesive sediment beds in a freshwater wave –dominated environment. *Limnology and Ocenography*, vol52 (2), pp 577-589.

7. Fodorpataki, L and Bartha, M. (2008) Differential sensitivity of the photosynthetic apparatus of a freshwater green alga and of duckweed exposed to salinity and heavy metal stress, *Photosynthesis energy from the sun.* Springer Netherlands publisher pp.1451-1454.

8. Gupta, P.K and Kumar. N (2014) Bio purification of Mine Wastewater through Aquatic Plants, *International Journal of Engineering and Technical Research* Vol 2(6), pp.186-188,

9. Hoek, C. and Mann, D. (1998) “Algae an introduction to physiology”, Cambridge university press, *Kluwer Academic Publishers*, Vol 35(4), pp.506.

10. Howells and Gwyneth. (1994) Water Qual Freshwater Fish. Gordon and Breach *science Publisher*, Vol. (6), pp.222.

11. Kathleen, R. Reiner. M and Gray. K. (2013) "Water Scarcity in Jaipur, Rajasthan, India." North-western University, Jal Bhagirathi Foundation.

12. Moore, L. and Thornton, K. (1988) The Lake and Reservoir Restoration Guidance Manual. 1st edn. *National Service Center for Environmental Publications,* pp.3-16,

13. Muhibbu-Din, O. I. Aduwo, A. O. and Adedeji, A. A. (2011) "Study of physiochemical parameter of effluent impacted stream in Obafemi Awolowo University, Ile-Ife, Nigeria." B.Sc (Hons) in Zoology Dissertation, Obafemi Awolowo University. *Journal of Academia and Industrial Research*, Vol (2), pp.1-28,

14. Nguyen, D.T. Paul, K. L. Glen, R.S. and Des, W.C. (2000) Toxicology and risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water. *Journal of Springer New York*. Vol (163), pp.113-185

15. Palmer, C.M. (1969) .A Composite rating of algae tolerating organic pollution, *Journal of Phycology* .vol (5), pp.78-82.

16. Patil, P.N. Sawant, D.V. Deshmukh, R.N. (2012) Physico-chemical parameters for testing of water, *International Journal Of Environmental Sciences*, Vol 3(3), pp.1195-1207.
17. Paswan, G. and Kumar, N. (2014) Bio purification of Waste Water through Algae, *International Journal of Engineering and Technical Research*, Vol-2(4), pp.71-72.
18. Phang, S. M. (2004), Handbook of microalgae culture, biotechnology and applied phycology, Journal *of applied phycology*, vol 6(2), pp.159-160.
19. Ponnuswamy, I. Madhavan, S. and Syed, S. (2013) "Isolation and Characterization of Green Microalgae for Carbon Sequestration, Waste Water Treatment and Bio-fuel Production." *International Journal of Bio-Science & Bio-Technology* .vol (5), pp.2
20. Prakash and Vijayvergia, R. (2008) "Recent Physico-Bio-Chemical approach to Lake Udaisagar, Udaipur (Rajasthan) with special reference to Water Pollution, Eutrophication & Lake type and a suggestive module for the Lake Environment Conservation*." 13th World Lake Conference. Rehabilitate the Lake Ecosystem: Global Challenges and the Chinese Innovations"*, Journal of World Lakes Database pp.1-5.
21. Prescott, G.W. (1956) How to Know the Fresh Water Algae, Michigan, *Springer-Verlag Publisher*, vol22 (3), pp.167-240.
22. Patrick, R. (1948) Factors effecting the distribution of diatoms. *Springer-Verlag Publisher*, Vol 14(8), pp.473-524.
23. Sigworth (1957) Control of odor and taste in water supplies. J.Amer. Water Wks. Assn.*Journal American Water Works Association*, Vol (49), pp.1507-1521.

24. Silvey, J. K. G. and Roach, A. W. (1959) "Laboratory Culture of Taste-and Odor-Producing Aquatic *Actinomycetes." Journal American Water Works Association*, Vol (59), pp. 20-32.

25. Sharma, K. P. Sharma, S. and Sharma, S. Sharma, P. K. and Swami, R. C. (2007) "Mansagar Lake: Past, Present & Future. In Proceedings of Taal*, The 12th World Lake Conference*, vol (1530), pp.1541-1543.

26. Sharma. Kumar, G. and Khan, S. A. (2013) "Bioremediation of Sewage Wastewater Using Selective Algae for Manure Production." *International Journal of Environmental Engineering and Management.* Vol (4), pp.573-580.

27. Trivedi, R. K. and Goel, P. K. (1986) Chemical and biological methods for water Pollution studies*, Environmental Publication, Karad, and Maharashtra*. Vol (6), pp.211-215.

28. Velan, M. and R, Saravanane. (2013) "CO2 Sequestration and Treatment of Municipal Sewage by Micro Algae." *International Journal of Innovative Technology and Exploring Engineering,* Vol (2), pp.307-310.

29. Xu, S. Nirmalakhandan,N. (1998) Use of QSAR models in predicting joint effects in multicomponent mixtures of organic chemicals. *Water Res, Elsevier Publisher*, vol (41), pp.2391-2399.